ANTI-INFLAMMATORY COMPOUNDS

Technical Field

The present invention relates to compounds having anti-inflammatory properties.

Background of the Invention

Inflammation at wound sites and sites of infection is often characterised by, *inter alia*, a strong infiltration of leukocytes at the site of inflammation. In particular, polymorphonuclear cells (PMN) are the predominant cell type recovered from the sites of inflammation, such as inflammatory joints (inflamed intraarticular and periarticular spaces) (Terkeltaub, 1992; Dieppe *et al.*, 1979).

Inflammation can be reduced by the action of, for instance, such anti-inflammatory agents as glucocorticoids, produced by the body in response to inflammation. One of the many actions carried out by glucocorticoids is the induction of lipocortin 1 (LC1), which itself inhibits arachidonic acid release and cell proliferation (processes usually associated with inflammation).

The reviewed experimental evidence (Flower and Rothwell, 1994) supports the concept that lipocortin (LC) 1 is a key mediator of many effects of glucocorticoids including the suppression of lipid mediator release (Cirino *et al.*, 1987) the inhibition of fever, (Carey *et al.*, 1990; Davidson *et al.*, 1991), paw oedema (Cirino *et al.*, 1989) and polymorphonuclear leukocyte (PMN) migration (Perretti *et al.*, 1993), the inhibition of the release of adrenocorticotrophic hormone (ACTH) (Taylor *et al.*, 1993) and other anterior pituitary hormones (e.g. Taylor *et al.*, 1993, 1995) and the inhibition of the induction by endotoxin of nitric oxide synthase (Wu *et al.*, 1995).

LC1 is a member of a super-family of proteins termed the annexins (reviewed by Raynal and Pollard, 1994). Members of this protein group are identified by a common structural motif comprising four repeating subunits (in some members of the family, eight repeating subunits). Whilst this core domain is highly conserved amongst

However, the studies carried out by Croxtall and colleagues (Croxtall *et al.*, 1998) was based upon an *in vitro* assay where cell division was measured.

Surprisingly, it has now been found that the *in vivo* anti-inflammatory properties of LC1 are contained within a different part of the N-terminal amino acid sequence of LC1, specifically $LC1_{2-6}$ (N-acetyl $LC1_{2-6}$ = AMVSE).

Summary of the Invention

According to the present invention, there is provided a compound comprising the amino acid sequence AMVSE, wherein said compound does not comprise the amino acid sequence EQEYVQTV.

Also provided by the present invention is a pharmaceutical composition which comprises a compound comprising the amino acid sequence AMVSE, wherein said compound does not comprise the amino acid sequence EQEYVQTV, and which further comprises one or more pharmaceutically acceptable excipients. Examples of such excipients include phosphate buffered saline (PBS) at, for example, 0.1 M, pH 7.4, $NaHCO_3$ at, for example, 0.2 M and other such physiologically acceptable fluids.

The present invention also provides the use of a compound comprising the amino acid sequence AMVSE, wherein said compound does not comprise the amino acid sequence EQEYVQTV, in the manufacture of a medicament for inhibiting leukocyte migration, or treating or preventing inflammation and/or inflammatory response/disease.

Yet further provided by the present invention is a method of inhibiting leukocyte migration, or treating or preventing inflammation and/or inflammatory response/disease, comprising administering to an animal an effective amount of a compound comprising the amino acid sequence AMVSE, wherein said compound does not comprise the amino acid sequence EQEYVQTV.

The present invention may employ any compound comprising the amino acid sequence AMVSE provided it does not comprise the amino acid sequence EQEYVQTV. Preferably, the compound is a polypeptide. The polypeptide may be acyclic or cyclic.

Figure 2 illustrates (as a bar chart) the degree of inflammatory response (as measured by PMN migration) generated by the *in vivo* activity of 66 nmol lipocortin 1-derived peptides with reference to Figure 1 (Scramble = $LC1_{2-6}$ (Ac-SVEMA); Ac = acetyl).

The *in vivo* anti-inflammatory properties of the amino acid sequence AMVSE were demonstrated by preparing fragments of N-acetyl $LC1_{2-26}$ (AMVSEFLKQAWFIENEEQEYVQTVK) and testing them in an animal model of inflammation. The *in vivo* animal model provided evidence that whilst N-acetyl $LC1_{2-12}$ (AMVSEFLKQAW) was active in the model, $LC1_{13-25}$ (FIENEEQEYVQTV) was not (data not shown). When AMVSE and $LC1_{7-12}$ (FLKQAW) were tested, the former was active whereas the latter was not. A scrambled version of AMVSE (namely, SVEMA) was also found to be inactive.

The experiments described herein clearly indicate that the biological properties of lipocortin 1 (LC1) differ in *in vivo* inflammatory models compared to the *in vitro* A549 model.

The compounds used in the present invention are preferably prepared for use as pharmaceuticals. The polypeptides may be administered by any suitable route including oral or parenteral administration. Pharmaceutical compositions which comprise the compounds described typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally or alternatively, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. The polypeptide-containing compositions are preferably administered in combination with pharmaceutically acceptable excipients such as 0.1 M PBS (pH 7.4), 0.2 M $NaHCO_3$ or other such pharmaceutically acceptable fluids.

Typically, the compositions contemplated are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in, for example, liposomes.

Experimental Protocol

Animals

Male Swiss Albino mice (20-22 g body weight) were purchased from Interfauna (CFLP strain; Huntingdon, Cambridgeshire, UK) and maintained on a standard chow pellet diet with tap water *ad libitum*. Animals were used at least one week after arrival.

Mouse Air-Pouch Model

Air-pouches were formed on the back of the mice by subcutaneous (s.c.) injection of 2.5 ml of air on day 2 and day 5. Three days after the last air-injection (6-day-old air-pouches) 1 mg of zymosan (in 0.5 ml of sterile saline) was injected locally (Perretti *et al.*, 1996). Zymosan was previously boiled for 30 min in phosphate buffered solution (PBS), extensively washed in the same medium and stored at –20°C prior to use.

Four hours after the local injection of zymosan, mice were killed by CO_2 exposure and the air-pouches washed with 2 ml of PBS containing ethylenediaminetetracetic acid sodium salt (EDTA; 3 mM) and heparin (25 U/ml). Lavage fluids (essentially the entire 2 ml were consistently recovered) were centrifuged at 200 *g* for 10 min at 4°C and cell pellets were resuspended in 2 ml of PBS/EDTA + heparin. The number of PMN was determined, using a Neubauer haematocytometer, after staining (1:10 dilution) with Turk's solution (crystal violet 0.01 % w/v in acetic acid 3% v/v).

Peptides

All peptides were synthesised following conventional solid phase technique by The Advanced Biotechnology Centre, Charing Cross Westminster Medical School (London, UK) and purifed by high liquid performance chromatography. All peptides were more than 95% pure.

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